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(54) PRODUCTION OF ANTIBODIES FROM TRANSGENIC ANIMALS

ERZEUGUNG VON ANTIKÖRPERN AUS TRANSGENEN TIEREN

PRODUCTION D'ANTICORPS A PARTIR D'ANIMAUX TRANSGENIQUES

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(56) References cited:
EP-A- 0 264 166
WO-A-88/00239
WO-A-88/01648
WO-A-88/05077
WO-A-88/10118

- **TIBTECH, vol. 5, 1987; A.J. CLARK et al., pp. 20-24/**
- **TIBTECH, vol. 5, 1987; R.B. CHURCH, pp. 13-19/**
- **SCIENCE, vol. 240, 1988; R. JAENISCH, pp. 1468-1474/**
- **TRENDS IN GENETICS, vol. 1, 1985; F.W. ALT et al., pp. 231-236/**
- **NATURE, vol. 323, 1986; E. ROBERTSON et al., pp. 445-448/**
- **ADVANCES IN GENETICS, vol. 24, 1987; G. SCANGOS et al., pp. 285-316/**

EP 0 438 474 B1

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Another possibility involves removal from the animal of immunoglobulin-producing cells generated by the animal after insertion of genetic material, followed by in vitro selection of cells producing an immunoglobulin of interest. The immunoglobulin can then be produced in vitro from the selected cells in known manner.

It has been found that a transgenic animal can produce chimaeric or foreign immunoglobulin (derived from inserted DNA) in response to an immunogen subsequently introduced to the animal. Accordingly, by introducing foreign, eg human, DNA encoding for substantially the entire species specific regions of an immunoglobulin it may be possible to stimulate the animal to produce foreign immunoglobulin to any antigen introduced to the animal. The transgenic animal could thus provide a highly useful, convenient and valuable source of human immunoglobulins to a large range of antigens.

It is thought that it may be important for the inserted DNA to be integrated in proximity on the genome for successful rearrangement. The inserted DNA may thus be in the form of DNA cloned into prokaryotic vectors such as plasmids and cosmids. Multiple plasmids or cosmids may also be used, but it is probably necessary for these to integrate in proximity on the genome. It may also prove possible to insert larger DNA fragments by using yeast artifical chromosome vectors (see Burke, D T, Carle, G F and Olson, M V (1987) "Cloning of large segments of exogenous DNA into yeast by means of artifical chromosome vectors" Science, 236, 806-812), or by introduction of chromosome fragments (see Richer, J and Lo, C W (1989) "Introduction of human DNA into mouse eggs by injection of dissected human chromosome fragments" Science 245, 175-177). Vertebrate chromosome or DNA fragments may also be used as the source of the inserted DNA.

The inserted DNA may be introduced to the host in conventional manner, for example by injection or other procedures into fertilised eggs or embryonic stem cells.

It may be convenient to use a host animal that initially does not carry genetic material encoding immunoglobulin constant regions so that the resulting transgenic animal will use only the inserted foreign genetic material when producing immunoglobulins. This can be achieved either by using a naturally occuring mutant host lacking the relevant genetic material, or by artificially making mutants eg in cell lines ultimately to create a host from which the relevant genetic material has been removed.

Where the host animal carries genetic material encoding immunoglobulin constant regions, the transgenic animal will carry the naturally occuring genetic material and the inserted genetic material and will produce immunoglobulins derived from the naturally occuring genetic material, the inserted genetic material and mixtures of both types of genetic material. In this case the desired immunoglobulin can be obtained by screening hybridomas derived from the transgenic animal, eg by exploiting the phenomenon of allelic exclusion of antibody gene expression or differential chromosome loss.

In a further aspect the present invention produces a transgenic animal, particularly a non-human mammal, which has had inserted into its germline DNA that includes a nucleic acid segment coding for at least part of an immunoglobulin of human origin which includes a gene segment coding for a human immunoglobulin heavy chain region wherein said gene segment is not in fully rearranged form, such that the DNA is rearranged in the animal to encode a repertoire of immunoglobulins with part or parts derived from the inserted DNA and expressed in cells or suitable body fluid of the animal.

The invention also includes within its scope an immunoglobulin obtainable from a transgenic animal in accordance with the invention or produced by the method of the invention.

In one example, lines of transgenic mice were established, carrying a DNA segment introduced into their germline that contains germline-configuration immunoglobulin VH genes, some of the D segments, and all the JH and Cmu gene segments. One of the VH genes, all the JH segments and the exons encoding the secreted heavy-chain constant region of IgM antibody were of human origin, with the remaining material being of mouse origin. The gene segments undergo productive VH-D-JH joining in the lymphoid tissue of the transgenic mice, with resultant synthesis of human/mouse chimaeric IgM antibody in serum.

Following immunisation, hybridomas have been established by fusion between spleen cells from the transgenic mice with the NSO myeloma. Many of the hybrids secerete human chimaeric IgM monoclonal antibodies. These lines of transgenic mice can therefore be used for the production of chimaeric human antisera or monoclonal antibodies.

Further, the mice make a response following immunisation with human antigens, producing chimeric antibodies to introduced antigen, and this approach should therefore also prove useful for the production of a repertoire of conventional human or chimaeric human antibodies directed against human as well as heterologous antigens, as the transgenic mice will not be tolerant to human antigenic determinants.

In another example, transgenic mice carrying exclusively human VH, D, JH and C mu sequences were produced by injecting into fertilised mouse eggs cosmids containing human VH genes, D segments, J segments and the C mu constant region. Resulting mice produced between 10 and 100 ug/ml antibody containing human mu chains in their serum, with mouse IgM being present at about 200 ug/ml.

The invention will be further described, by way of illustration, in the following Examples which refer to the accompanying drawings, in which:

The IgH Enhancers:

Part of the human IgH enhancer is included within the BglII fragment containing the JH cluster. A full copy of the mouse enhancer is included within the 1 Kb XbaI fragment.

The Switch region and Cmu region:

The 7.5 Kb XbaI fragment of human DNA includes the mu switch region and exons 1 to 4 of the mu heavy-chain constant region. The mu membrane exons and the bulk of the intron between the Cmu4 exon and the CmuMI membrane exon are provided by a 2.5 Kb HindIII-SphI fragment of the mouse mu CH gene in which the SphI site was converted to a BglII site by use of linkers.

The Transgenic mice

Plasmid DNA was linearised with BglII, purified after electrophoresis in an agarose gel and injected into the male pronucleus of fertilised eggs of C57BL/6J x CBA/Ca mice as previously described [Reik et al. (1987) Eur. J. Immunol. 17, 465-469]. Southern blot analysis of tail DNA revealed that 12 of the 32 mice born carried the mini-locus. Most subsequent work was performed on offspring of three founder mice - Hlg 17, 19 and 29 all of which carry a low number (2-5) of copies of the mini-locus.

Serum Assays

Serum of the founder mice was tested by ELISA for the presence of antibody containing antigenic determinants characteristic of human IgM. The uniminunised transgenic mice proved to contain tween 10 and 100 ug/ml of chimaeric human IgM in their serum. Immunofluorescence analysis of lymphocytes in peripheral blood also revealed the presence of cells staining with biotinylated species-specific anti-human Ig M antibody and fluorescein-conjugated streptavidin.

Hybridomas from transgenic mice

Transgenic mice were immunised intraperitoneally with either human red blood cells or sheep red blood cells. Spleens were removed at various times after immunisation, fused with the NSO myeloma and hybrids selected in HAT medium. Many of these hybrids made chimaeric human IgM as revealed by ELISA assay.

DNA Rearrangement of the mini-locus

Southern blot analysis of DNA from tissues from the transgenic mice as well as from the hybridomas revealed that there is a high frequency of DNA rearrangements within the mini-Ig locus in the lymphoid tissue of the transgenic mice.

DNA from tissues or hybridomas established from the transgenic mice was digested with EcoRI and hybridized with a human IgH enhancer probe (BalI-BglII fragment) that hybridizes to the region between the human J6 element and the mouse IgH enhancer in the mini-locus. The results of Southern blot analysis of the DNA are shown in Figure 2. The sizes in Kb of marker fragments are given in the Figure.

Transcription of the mini-locus

Cytoplasmic RNA (5 ug) from the NSO fusion partner or from hybridomas from the transgenic mice was probed with human Cmu, human VH26 or mouse VH186 probes The results of Northern blot analysis of the cytoplasmic RNA are shown in Figure 3, which reveals that the hybridomas contained mRNA that hybridized with probes for human mu as well as for either or both of the VH26 or VH186 V genes. Thus both the human VH26 and mouse VH186 are able to rearrange and thus create a cell-line that secretes a chimaeric human IgM antibody.

Antibody secretion by hybridomas from the transgenic mice

Protein production by cloned hybridoma cell-lines was analysed by use of biosynthetic labelling with L-[^{35}S] methionine and subsequent purification with anti-human mu antiserum. In particular, cells were incubated overnight in medium containing L-[^{35}S] methionine and IgM antibody purified from the culture supernatant by immunoprecipitation and an anti-human mu antiserum. The purification from the supernatants of the transgenic hybridomas 35.5 and 24a was performed in the presence of a large excess (50ug) of non-radioactive, purified mouse monoclonal IgM antibody (B1-8) as indicated by "+" in Figure 4. As seen using the mouse IgM secreting cell-line, the anti-human mu antiserum

A shift of the profiles to the right (increased fluorescence) denotes a positive stain that can only been seen for antibodies containing human mu heavy chains and mouse kappa light chains but not for antibodies containing mouse mu heavy chains or mouse gamma heavy chains (not shown).

Table 1

Antibody in the body fluids of IgA2, lambda 1-mice					
	IgA2 anti NP Ab			Total kappa bearing Ab	
Mouse	Serum	Milk	Colostrum	Milk	Colostrum
TG1	10	0 6	2 1	960	600
TG2	6.3	0.56	1.4	1000	420
TG3	11.3	1.3	ND	735	ND
TG4	7.3	0.8	1.4	780	660
TG5	30	7.6	10.0	1250	ND
TG6	34.6	5.0	10.0	500	600
TG7	6.3	0.64	0.93	780	600
Control	0	0	0	1136	660
ND, not determined					

All concentration in ug/ml

Claims

1. A method of producing an immunoglobulin, comprising inserting into the germline of a non-human animal DNA that includes a nucleic acid segment coding for at least part of an immunoglobulin of human origin which includes a gene segment coding for a human immunoglobulin heavy chain region wherein said gene segment is not in fully rearranged form, such that the DNA is rearranged in the animal to encode a repertoire of immunoglobulins with part or parts derived from the inserted DNA and expressed in cells or body fluid of the animal; and obtaining immunoglobulin from cells or suitable body fluid of the animal.

2. A method of producing an immunoglobulin to a particular antigen, comprising producing a transgenic non-human animal by inserting into the germline of the animal DNA of foreign origin coding for at least part of an immunoglobulin of human origin which includes a gene segment coding for a human immunoglobulin heavy chain region, such that said DNA of foreign origin undergoes rearrangement or mutation in the lymphoid tissue of the transgenic animal to produce a variety of rearranged genes that encode immunoglobulins, immunglobulin fragments or immunoglobulin chimeric molecules such that, following challenge of the transgenic animal with a particular antigen, the immunoglobulin to the antigen that is encoded by the rearranged or mutated DNA is expressed in cells or body fluid of the animal; and obtaining immunoglobulin from cells or suitable body fluid of the animal.

3. A method according to claim 1 or 2, wherein polyclonal antiserum comprising the immunoglobulin is obtained from the animal.

4. A method according to claim 1 or 2, wherein monoclonal antibody comprising the immunoglobulin is produced using cells obtained from the animal.

5. A method according to claim 1 or 2, wherein the immunoglobulin is produced in a body fluid or secretion of the animal.

6. A method according to claim 1 or 2, wherein the immunoglobulin is produced in vitro from cells obtained from the animal.

7. A method according to any one of the preceding claims, wherein the inserted DNA encodes substantially the entire species specific regions of an immunoglobulin.

8. A method according to any one of the preceding claims, wherein the transgenic animal is a mouse.

10. Verfahren nach einem der vorangehenden Ansprüche, wobei die DNA durch Injektion oder andere Verfahren in befruchtete Eier oder embryonale Stammzellen inseriert wird.

11. Verfahren nach einem der vorangehenden Ansprüche, wobei das Tier zunächst kein genetisches Material trägt, das konstante Bereiche von Immunglobulin codiert.

12. Immunglobulin fremden Ursprungs, erhältlich durch das Verfahren nach einem der vorangehenden Ansprüche.

13. Transgenes nicht-menschliches Tier, in dessen Keimlinie DNA inseriert ist, die ein mindestens einen Teil eines Immunglobulin menschlichen Ursprungs codierendes Nucleinsäuresegment umfaßt, das ein Gensegment einschließt, welches einen Bereich der schweren Kette eines menschlichen Immunglobulins codiert, wobei das Gensegment nicht in vollständig rearrangierter Form vorliegt, wobei die DNA in dem Tier rearrangiert wird, so daß sie eine Anzahl von Immunglobulinen mit einem Teil oder Teilen, die von der inserierten DNA stammen, codiert, und in Zellen oder geeigneter Körperflüssigkeit des Tieres exprimiert wird.

Revendications

1. Procédé de production d'une immunoglobuline, comprenant l'insertion dans la lignée germinale d'un animal non-humain d'un fragment d'ADN qui contient un segment d'acide nucléique codant pour au moins une partie d'une immunoglobuline d'origine humaine, incluant un segment de gène codant pour une région de chaînes lourdes d'immunoglobuline humaine, dans lequel ledit segment de gène n'est pas sous une forme entièrement réarrangée, tel que l'ADN soit réarrangé dans l'animal.pour coder un répertoire d'immunoglobulines avec une ou des parties dérivée(s) de l'ADN inséré et exprimée(s) dans les cellules ou un fluide corporel de l'animal ; et l'obtention de l'immunoglobuline à partir des cellules ou du fluide corporel approprié de l'animal.

2. Procédé de production d'une immunoglobuline dirigée contre un antigène particulier, consistant à produire un animal transgénique non-humain en insérant dans la lignée germinale de l'animal un fragment d'ADN d'origine étrangère codant pour au moins une partie d'une immunoglobuline d'origine humaine incluant un segment de gène codant pour une région de chaîne lourdes d'une immunoglobuline humaine, tel que ledit ADN d'origine étrangère subisse un réarrangement ou une mutation dans le tissu lymphoïde de l'animal transgénique pour produire une variété de gènes réarrangés qui codent des immunoglobulines, des fragments d'immunoglobulines ou des molécules chimériques d'immunoglobulines tel que, après provocation de l'animal transgénique avec un antigène particulier, l'immunoglobuline dirigée contre l'antigène qui est codée par l'ADN réarrangé ou ayant subi une mutation s'exprime dans les cellules ou un fluide corporel approprié de l'animal.

3. Procédé selon la revendication 1 ou 2, dans lequel l'antisérum polyclonal comprenant l'immunoglobuline est obtenu de l'animal.

4. Procédé selon la revendication 1 ou 2, dans lequel l'anticorps monoclonal comprenant l'immunoglobuline est produit en utilisant les cellules obtenues de l'animal.

5. Procédé selon la revendication 1 ou 2, dans lequel l'immunoglobuline est produite dans un fluide corporel ou une sécrétion de l'animal.

6. Procédé selon la revendication 1 ou 2, dans lequel l'immunoglobuline est produite in vitro à partir de cellules obtenues de l'animal.

7. Procédé selon l'une quelconque des revendications qui précèdent, dans lequel l'ADN inséré code essentiellement pour les régions spécifiques de la totalité des classes d'une immunoglobuline.

8. Procédé selon l'une quelconque des revendications qui précèdent, dans lequel l'animal transgénique est une souris.

9. Procédé selon l'une quelconque des revendications qui précèdent, dans lequel l'ADN inséré comprend un plasmide ou un cosmide ; des plasmides ou des cosmides multiples ; un chromosome artificiel de levure ; ou un chromosome de vertébré ou des fragments d'ADN.

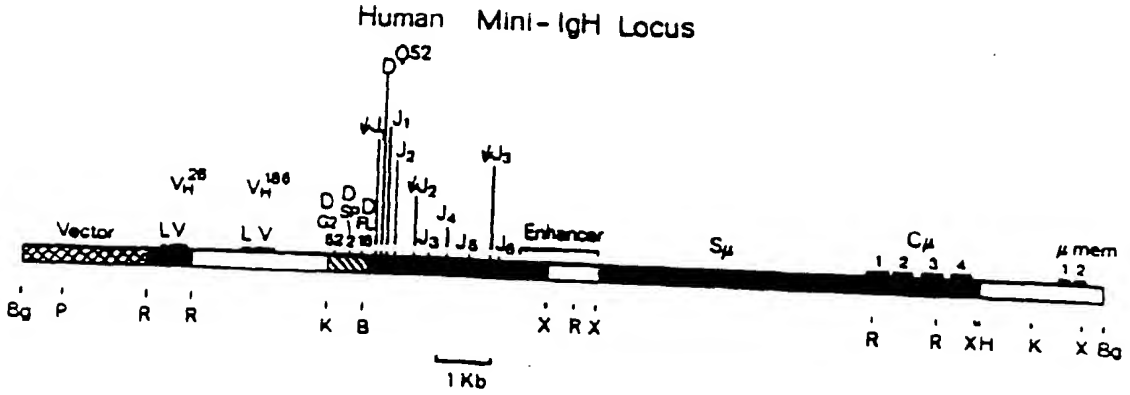
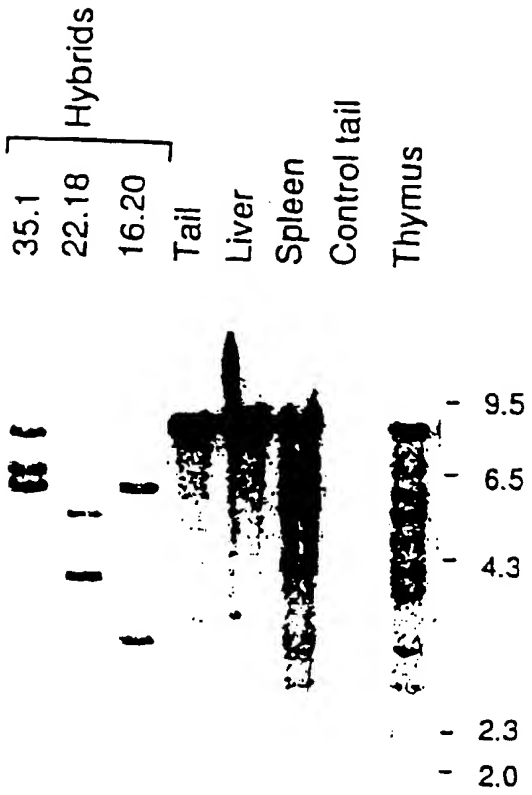


Fig. 1



EcoRI

Fig. 2

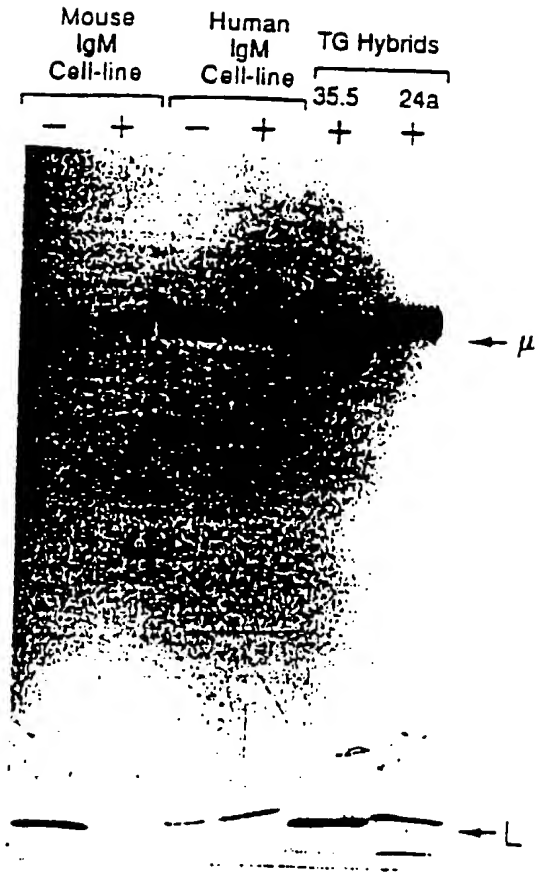

Mouse IgM Cell-line
Human IgM Cell-line
TG Hybrids
35.5
24a
− + − + + +
μ
L

Fig. 4

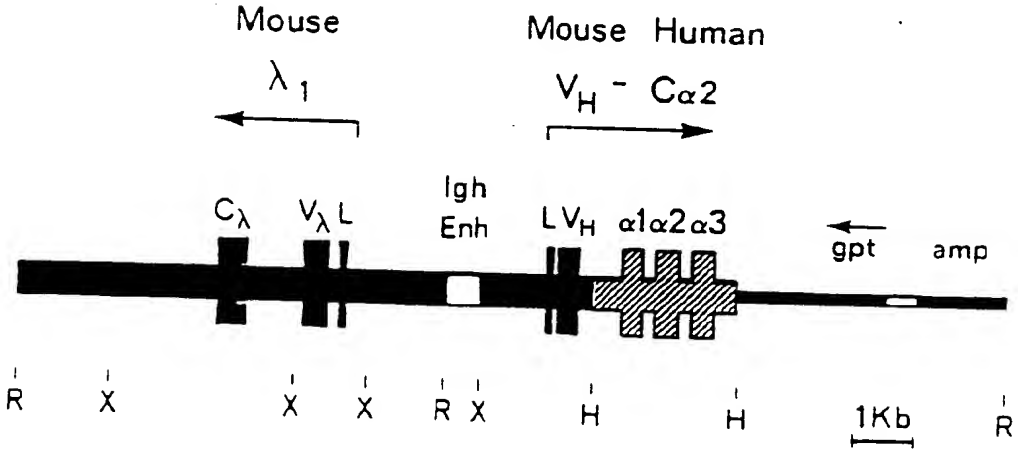
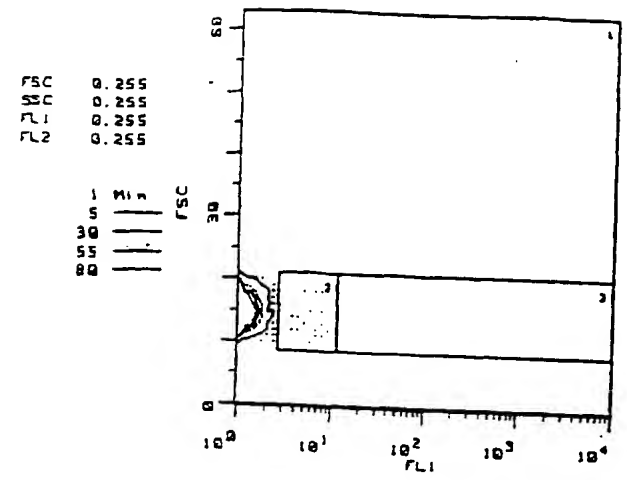

Mouse
λ 1
Mouse Human
V_H - Cα2
C_λ
V_λ L
Igh Enh
L V_H
α1α2α3
gpt
amp
R X X X R X H H 1Kb R

Fig. 5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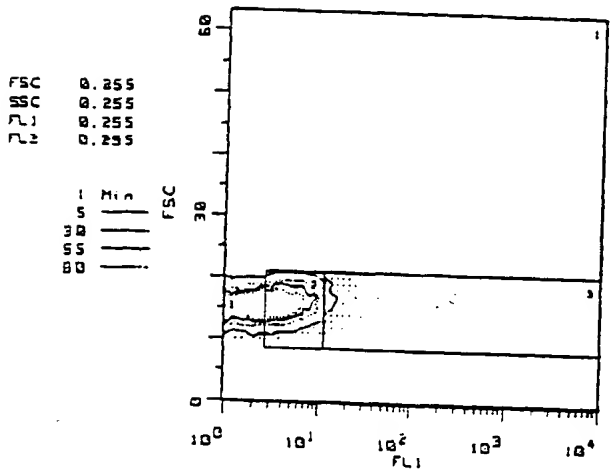
SSC 0.255
FL1 0.255
FL2 0.255
1 Min
5
30
55
80
FSC
60
30
0
1
2
3
10^0
10^1
10^2
10^3
10^4
FL1

A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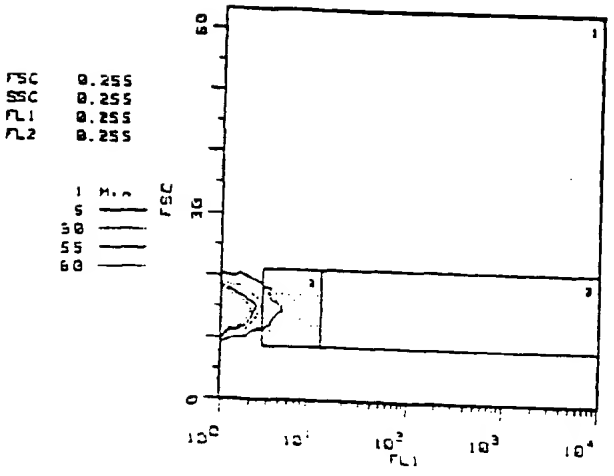
FL1

B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SSC 0.255
FL1 0.255
FL2 0.255
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FL1

C

Fig. 7